Hydrogen isotope systematics of hair: archeological and forensic applications

Zachary D. Sharp[a]*, Viorel Atudorei[a], Héctor O. Panarello[b], Jorge Fernández[b]✠, Chuck Douthitt[c]

[a] *Department of Earth and Planetary Sciences, Northrop Hall, University of New Mexico, Albuquerque, NM 87131, USA*
[b] *Instituto de Geocronología y Geología Isotópica, Pabellón INGEIS, Ciudad Universitaria, (1428) Buenos Aires, Argentina*
[c] *ThermoFinnigan, 9412 Rocky Branch Drive, Dallas, TX75243, USA*

Abstract

The hydrogen isotope systematics of hair have been explored and several applications demonstrate the type of information that can be recovered from archeological and forensic hair samples. Experiment with modern hair demonstrate that sources of protein-based hydrogen in keratin are food and water. On the basis of a deuterium-enriched drinking study, it was found that $\sim 31\%$ of hydrogen in human hair is derived from ingested water. At least 9% of total hydrogen in human hair is isotopically exchangeable with water or water vapor at 25 °C. Nearly complete exchange occurs in a matter of hours. The δD value of body water is approximately $17 \pm 10‰$ ($n=7$) more negative than human hair, although there is much scatter in data for modern hair, which is clearly related to variability of the isotopic composition of ingested food and water. Archeological hair samples were analyzed from (i) a 370 year old Incan mummy sacrificed at 5300 m altitude in Argentina (Geoarchaeology 14 (1999) 27) and (ii) from a Woolly Mammoth from Siberia. High spatial resolution data along the length of hair from the mummy indicate seasonal variations in carbon, nitrogen and hydrogen isotope ratios. No hydrogen isotope anomalies are seen in the youngest hair at the base of the scalp, indicating that the individual probably lived at ~ 1600 m, and spent less than a week at high altitudes before death. The mammoth also preserves a seasonal signal, suggesting hair growth rates of ~ 33 cm/year, faster than human hair, but slower than horse tail hair. The limited isotopic range across seasons for the mammoth is consistent with a north-south migration on the order of 1000 km.
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1. Introduction

Interpretation of the significance of the hydrogen isotope composition of animal-based organic matter is complicated by a number of factors [27]. In contrast to plants, which essentially derive all of their hydrogen from water, organically bonded hydrogen in animals is derived from both food and water [7,8,26]. A further complication is that hydrogen bound to oxygen and nitrogen is easily exchangeable [5]. This same problem exists for plants, but conversion of plant tissue to cellulose nitrate removes most non-carbon bound hydrogen, thus minimizing the problem [6,26]. Chitin and keratin in phases such as hair, feathers, baleen, hoof, etc. are more resistant than cellulose to nitration, so that the problem of exchangeable hydrogen is not easily eliminated (e.g., [27]). In spite of the complications, there are a growing number of studies that have successfully used hydrogen isotope ratios of bird feathers to trace their migration patterns [2,12,15,19], and the applications continue to grow. In this communication, the feasibility of applications to hair is explored, including evaluating fractionation factors, extent and kinetics of isotopic exchange, and practicality of use in archeological and forensic samples.

In contrast to feathers, there have been far fewer isotope-based studies of hair, even though it is very

* Corresponding author. Fax: +1-505-277-8843.
✠ Deceased 24 December 2001.
  E-mail address: zsharp@unm.edu (Z.D. Sharp).

commonly found at archeological sites and on mummi-fied samples [17]. Hair, composed of α-keratin, is very resistant to diagenesis, although it can be degraded by some fungi ([17], and references therein). Hair has the added advantage over other archeological materials in that there is a very well defined time component-variations in chemical compositions that can be measured along the length of a single hair, so that temporal variations at a daily or weekly level can be assessed [31]. Carbon, nitrogen and sulfur isotope ratios of bulk hair have been shown to be diagnostic of diet [9,14,17,20–22,30,31,33], but we are not aware of any hydrogen isotope studies of hair.

2. Samples

Samples of hair and body water (urine) were collected from seven individuals from around the world in order to obtain an average fractionation between body water and hair. In order to determine the percentage of body water that is incorporated in hair, one of us (Z. Sharp) ingested water with a δD value of +1000‰ (prepared by adding 0.16 ml of 'heavy water' D_2O per liter of water). Urine and shaved beard stubble were collected daily and on a two-day basis, respectively. Before each dry shaving, the face was thoroughly rinsed and dried. Beard stubble received no further treatment prior to isotopic analysis.

Two ancient samples were analyzed. The Inca mummy from the SW side of Mount Aconcagua in Argentina [9], radiocarbon-dated to 370 ± 70 yr B.P., was a 7–10 year old boy buried as part of a ritual offered to the deities. The burial was discovered at ca. 5300 m altitude by a 1985 expedition organized by the Universidad Nacional de Cuyo and the Club Andinista de Mendoza [9]. Hair 20 cm in length extending from the scalp were sampled at $\sim$5mm intervals.

We sampled millimeter-long sections of a single 20 cm long hair of a Woolly Mammoth from the Yuribei Locality on the Gidan Peninsula, West Siberia, that was donated to the Smithsonian Institution (R. Purdy, pers. comm., 2002).

3. Methods

Hydrogen isotope ratios were determined using a continuous flow high temperature reduction technique [29]. One μl aliquots of urine are injected into a helium stream through a heated septum. The vaporized sample is reduced to CO and H_2 while passing over a graphite column heated to 1450 °C (Finnigan MAT TC EA). Reactant gases are purified by passage through a 5A mol sieve GC column heated to 100 °C, through a Finnigan MAT CONFLO II interface/open split for helium dilution and into a Finnigan MAT Delta XL Plus mass spectrometer. Solids are wrapped in silver foil and

Table 1
δD values of body water (measured as urine) and beard stubbles measured over and after the interval during which water of 1000‰ was ingested. Heavy water ingestion began on day 0 and ended on day 19 (n.d. not determined)

Day	δD urine (SMOW)	δD hair (SMOW)
0	−58	−83
0.5	−52	n.d.
1	−20	n.d.
1.5	20	n.d.
2	55	n.d.
3	108	n.d.
4	139	−83
5	172	n.d.
6	199	−84
7	220	n.d.
8	250	−84
9	274	n.d.
10	297	−63
11	294	n.d.
12	287	−37
13	283	n.d.
14	278	−33
15	292	n.d.
16	298	−16
17	295	n.d.
18	n.d.	−5
19	297	n.d.
20	289	14
21	337	n.d.
22	n.d.	n.d.
23	279	n.d.
24	n.d.	17
25	n.d.	n.d.
26	n.d.	17
27	n.d.	n.d.
28	n.d.	17
29	235	n.d.
30	n.d.	n.d.
31	211	n.d.
32	n.d.	n.d.
33	n.d.	n.d.
34	152	n.d.

admitted into the combustion chamber using a Carlo Erba AS 200-LS autosampler. Data are reported in conventional delta notation [18] relative to SMOW. Liquid measurements are calibrated relative to SMOW ($\delta D \equiv 0$‰), SLAP ($\delta D = -428$‰) and internal standard waters [3]. Solids measurements were calibrated relative to NBS 30 biotite ($\delta D = -65$‰) and numerous internally calibrated standards. Internal precision is $\sim \pm 1$‰ for liquids and ± 2‰ for solids. Total urine was analyzed and assumed to be equal to the δD values of body water. Urine is 96.5% water by weight, with the remainder being essentially salts. Nevertheless, we distilled a split of human urine and found that the δD value was identical (within 1‰) to that of the total urine, validating our assumption that urine is an accurate proxy for body water.
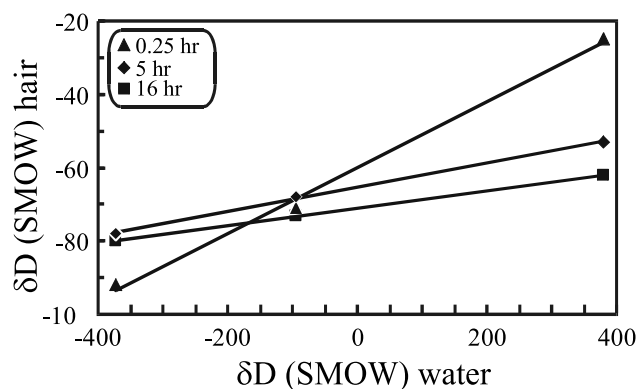
Fig. 1. Abundance of isotopically exchangeable hydrogen in human facial hair. Samples were equilibrated with waters of different compositions for between 2 and 3 weeks. Analyses were made immediately after removal from water (triangles), after re-equilibration in ambient air for 5 h in air (diamonds), and after re-equilibration in ambient air for 16 h (squares). The flatter slope of samples re-equilibrated with air for only 5 h demonstrates the rapid rate of exchange. Based on the maximum slope, our estimate for the abundance of exchangeable hydrogen as a percentage of total hydrogen is on the order of 9%.

4. Results

4.1. Exchangeable hydrogen

Non-carbon bound hydrogen (i.e., oxygen or nitrogen bound hydrogen) is exchangeable with liquid water and atmospheric water vapor. In order to estimate the fraction of exchangeable hydrogen in total hydrogen in hair-keratin, exchange experiments with artificially light and heavy water were conducted at 25 °C following previously described methods [2,25]. Human beard stubble was exchanged with waters of different isotopic compositions (−374‰, −95‰, +380‰) for 2 to 3 weeks at room temperature. In the case of feathers, it has been shown that exchange rates and final δD values are independent of whether the feather was equilibrated with water or water vapor [2]. The experiments of the present study were therefore made by immersing stubble into vials of water.

In bird feathers, complete exchange of exchangeable hydrogen occurred over a period of several weeks. The exchange rate for human facial hair appears to be much faster. Stubble was analyzed immediately after removal from water, after being allowed to re-equilibrate with air at room temperature for 5 h and overnight. Fig. 1 shows that even in a period of 5–12 h, a significant proportion of exchangeable hydrogen had reequilibrated with air moisture, trending towards a value that would be constant if the hydrogen isotopic ratio of atmospheric moisture were invariant (the latter assumption may only be true for a period of days, and therefore we performed sequential measurements in rapid succession). That is, the slope on a δD_{water} vs. δD_{hair} plot decreases over a period of hours as exchangeable hydrogen re-equilibrates with ambient atmospheric moisture. The
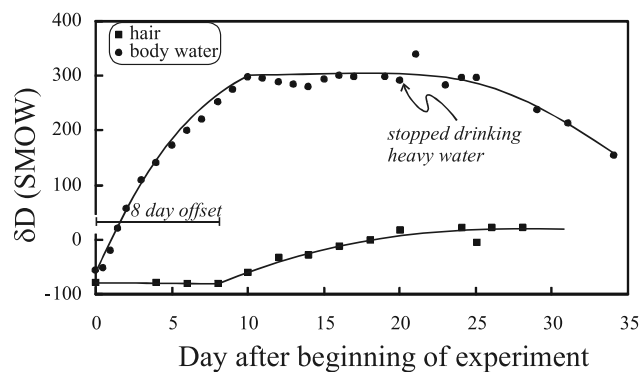

Fig. 2. δD values of urine over time after ingestion of +1000‰ drinking water. The δD values of urine increased by 350‰, while δD values of beard stubbles only increased by 100‰. The isotopic response of exposed stubble relative to that of urine was delayed by 8 days.

percentage of exchangeable hydrogen in total hydrogen was calculated using a simple mass balance equation

$$\delta D_{hair} = (1-f)\delta D_n + f\delta D_e \qquad (1)$$

and

$$\delta D_{hair} = (1-f)\delta D_n + f(\delta D_{water} - \varepsilon) \qquad (2)$$

where δD_{hair}, δD_n, δD_e and δD_{water} are the δD values of measured total hair hydrogen, non-exchangeable hair hydrogen, exchangeable hair hydrogen and water equilibrated with the sample, respectively. f is the fraction of exchangeable hair hydrogen and ε is the fractionation between non-exchangeable hair hydrogen and water $(\delta D_n - \delta D_W)$. From Eq. (2), it follows that the slope generated by a plot of δD_W vs. δD_T will express the fraction of exchangeable hydrogen f, or the hydrogen exchangeability in hair (Fig. 1). Samples which were analyzed immediately after being removed from water had 9.0% exchangeable hydrogen ($R^2 = 0.996$). Samples that were allowed to re-equilibrate with air for several hours had an apparent exchangeable hydrogen content of 3.3% and those left exposed to air overnight had only 2.3% exchangeable hydrogen. Similar to feathers [2], the total amount of exchangeable hydrogen in hair has a maximum value of ∼9% at 25 °C, but the exchange rate for hydrogen in human facial hair is much faster than for feathers.

4.2. Fraction of hydrogen from drinking water

In the experiment where an individual human adult drank water with a δD value of +1000‰, δD values of urine increased from an original value of −58‰ to a final value of +293‰ over 10 days, an overall 350‰ increase (Table 1, Fig. 2). The δD values of body water never approached the +1000‰ value of the enriched drinking water, because it constituted only a portion of

total ingested hydrogen in fluid and food. Maximum δD values of urine were reached after 10 days, after which no further changes were observed. Ingestion of high δD water was terminated after 19 days, after which δD values of urine began to slowly decrease toward their original value. Beard hair followed a similar pattern, but an 8 day delay is observed because new growth of hair takes about a week to travel from the subcutaneous follicle to exposure (Fig. 2). Maximum δD values were only +17‰, compared to an original value of −83‰, yielding a net 100‰ shift. Assuming a 9% exchangeable hydrogen component, the net change of non-exchangeable hydrogen is estimated to about 110‰ (100‰/0.91). The fraction of hydrogen in hair that derives biochemically from ingested water can be generated from Eq. (2) above, and is given by

$$\% \ H_2 \ \text{from water} = \frac{\Delta D_{\text{hair}}}{\Delta D_{\text{water}}} = \frac{110}{351} = 31\% \qquad (3)$$

This result is in agreement with a previous estimate for feathers (26–32%) and nails (27%) from quail [11]. The remaining ∼70% of hydrogen incorporated in hair derives from ingested food hydrogen. The δD values of food were varied and not measured.

Earlier studies have shown that the δD values of feathers correlates well with local meteoric water δD values [2,12,19]. Although most hydrogen in hair is biochemically derived from food, the δD values of local food sources should generally correlate well with local δD values of meteoric water, so that δD values of organic matter such as hair or feathers should also correlate with δD values of meteoric water. Exceptions to this relationship would be found in animals that migrate, or eat exotic food sources (e.g., marine-based diet) that are not isotopically related to local meteoric water.

4.3. Fractionation between body water and hair

Hobson and Wassenaar [12] made an empirical calibration for the hydrogen isotope fractionation between meteoric water and songbird feathers. Their best-fit linear regression to samples from 15 localities ranging from Alaska to the Gulf Coast is

$$\delta D_{\text{feather}} = 0.86 \ \delta D_{\text{meteoric water}} - 33‰ \qquad (4)$$

The correlation has a slope of less than 1, similar to the relationship found for $\delta^{18}O$ values of body water vs. meteoric water (e.g., [16]). In the case of oxygen isotopes, a slope of <1 is expected, as part of an animal's body fluid oxygen is obtained from atmospheric O_2 [1], which does not vary with latitude. However, there is no similar latitude-independent hydrogen isotope input for animals. The slope of 0.86 can be explained if 14% of the measured hydrogen was exchangable, in good agreement with experiments.
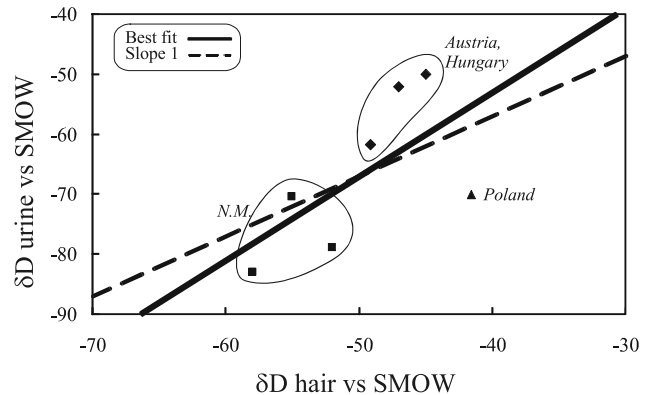

Fig. 3. δD values of hair vs. urine of people from New Mexico USA, Hungary, Austria and Poland. The data set is scattered due to different, non-local food sources, and is too small to make a highly accurate correlation between the δD values of body water and hair. An average ΔD(hair-body water)=17±10‰ is taken as a tentative fractionation.

The fractionation between hair and body water for *modern* humans was evaluated by analyzing the δD values of body water (urine) and hair from seven individuals. This empirical calibration is not an exact corollary to wild animal or prehistoric humans because contemporary people often consume foods that are not locally grown and may drink imported bottled fluids. While the δD value of urine will accurately reflect the δD of body water, the δD values of food and water intake could be temporally decoupled from that of body water. Individuals from regions around the world were sampled to minimize regional bias (Fig. 3). The average fractionation between body water and hair is 17‰ with a standard deviation of 9‰ (1σ). The large scatter in the data is probably due to food with δD values that are decoupled from local meteoric water values. The δD values of hair and body water are grouped for each geographic region. For example, the ΔD (hair-body water) value for the three samples from New Mexico are 22±6‰, whereas the two samples from Budapest are both 5‰. A constant offset of 17‰ is taken as a preliminary value for the fractionation between hair and body water. It is expected that the fractionation between water and food for ancient samples should be far more constant, as food sources would invariably be locally derived.

4.4. Application to ancient samples

4.4.1. Inca mummy

The δD, $\delta^{13}C$ and $\delta^{15}N$ values of the Inca mummy vary systematically as a function of length along the hair (Fig. 4). In previous studies the $\delta^{13}C$ value of the mummy's collagen was found to be −10.8‰ (PDB), consistent with a high maize (C4 plant) diet. The measured $\delta^{15}N$ value of +10.4‰ (AIR) is slightly higher than typical maize-based diets, which range from 9.0 to
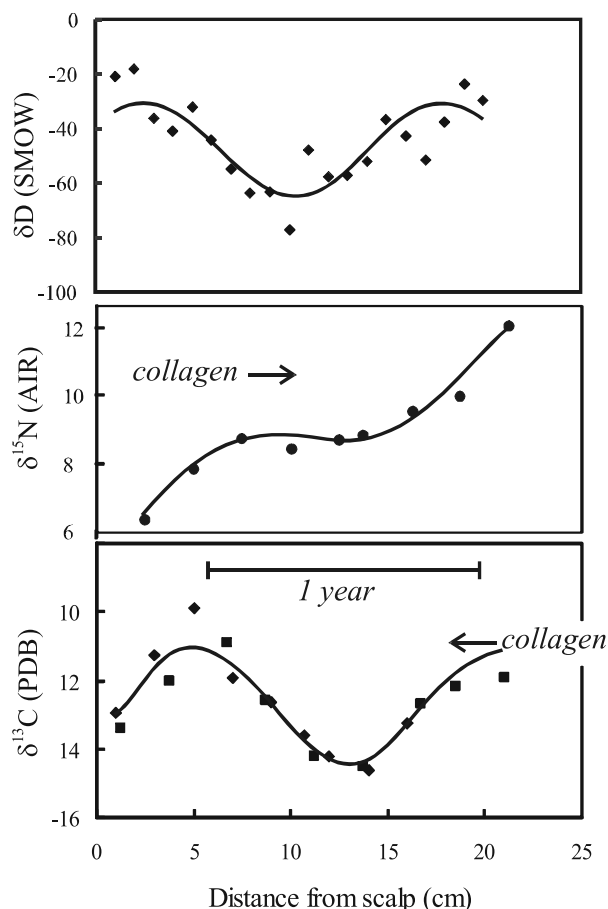
Fig. 4. δ^{13}C, δ^{15}N and δD values of hair as a function of distance from scalp for Inca Mummy and best-fit curves using non-linear sin function fitting procedures. For carbon and hydrogen the fit is to the form $y=a+b \times \sin (cx+d)$, where y is the measured δ value and x is distance in cm. Nitrogen data were fit to a curve of the form $y=a+b \times \sin (cx+d)+ex$, the extra term added to account for the increasing δ values with distance. For carbon, the best fit is obtained with the following coefficients: $a=-12.8$, $b=1.733$, $c=0.4067$, $d=5.69$, $R^2=0.838$. Assuming that the sinusoidal pattern is related to annual variations in diet (more maize in summer), the hair growth length is 15.4 cm. Hydrogen isotope data are best fit with the equation parameters $a=-40.4$, $b=21.17$, $c=0.262$ and $d=0.517$ ($R^2=0.701$). Refitting with the same c value (periodicity) as for carbon forces the same annual variation with only a slightly lower proportion of variance ($R^2=0.663$). The low δD values should correspond to winter and high δD values summer. Collagen values measured by Fernández et al. [9] are shown as arrows. Carbon data for hair by Fernández et al. [9] are shown as squares.
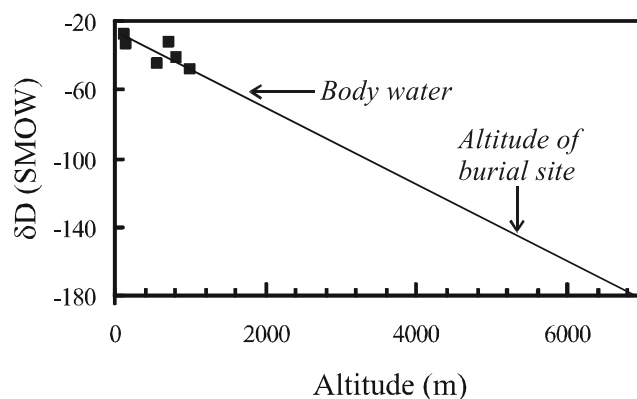

Fig. 5. δD values of meteoric water as a function of altitude. Black squares are published IAEA data for Argentina [13]; diagonal line is the altitude-δD relationship established by Poage and Chamberlain [24] worldwide. The average δD value for meteoric water determined from mummy hair corresponds to an altitude of <1600 m, far less than the 5300 m where the body was found. Had the boy lived at high altitude for more than ~1 week, the δD value of the hair at the base of the scalp would have been much lower.

9.9‰ [28,32]. The measured 10.4‰ δ^{15}N value falls in the range of 8 to 13‰ for samples of bone collagen from Inca-related Sausas [10]. The 10.4‰ value may indicate a mixed terrestrial/marine diet [9]. Variations in δ^{13}C values observed in the present study (Fig. 4) are in good agreement with those of Fernández et al. [9]. The variations are consistent with seasonally fluctuating importance of maize as part of the diet. The δ^{15}N values of hair are generally lower than for bone collagen (Fig. 4). The δ^{15}N values for hair do not indicate a strong marine component, supporting a previous interpretation that in the latter year of the boy's life, he had a mostly non-marine diet [9].

The δD, δ^{13}C and δ^{15}N values of the Inca mummy vary in a sigmoidal pattern as a function of length along the hair (Fig. 4). A statistical regression was applied to the carbon and hydrogen data for a non-linear sine function of the form $y=a+b \times \sin (c \cdot x+d)$ (where $y=\delta^{13}$C value of hair, x=length along hair). The sigmoidal variation in δ values is thought to be related to seasonal variations. The low δD values most likely correspond to winter months, where lower δ^{18}O and δD values of meteoric water are expected [4]. The carbon isotope variations are consistent with seasonally fluctuating importance of maize as part of the diet. Higher δ^{13}C values in summer suggest that more maize was consumed at this time. For carbon, the data are best fit with a periodicity of ~15.4 cm, in good agreement for a typical growth rate of hair of ~0.35 mm/day [9]. The best-fit periodicity for hydrogen isotope ratios is slightly shorter, but can be fit with the same periodicity with almost the same degree of correlation. The carbon data are preferred because they better define a sine function (Fig. 4).

The δD values of hair are surprisingly heavy, ranging from −60 to −20‰, with an average value of −44‰ (σ=15‰; n=20). Given an average fractionation of 17‰ between hair and body water, the δD values of body water would have been approximately −61‰. Assuming that the δD value of body water and meteoric water are similar, then the δD value of local meteoric water attending the growth of the Incan mummy's hair would have been around −61‰. Modern local meteoric water values as a function of elevation are shown in Fig. 5 based on the GNIP data base for Northern Argentina [13] and extended to higher altitudes using a correlation
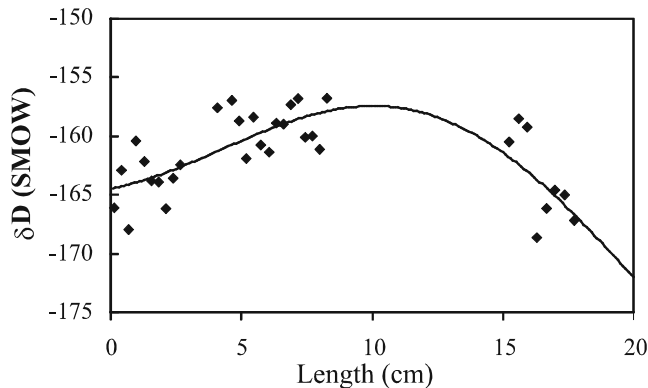
Fig. 6. δD values of mammoth hair as a function of distance along hair. The best fit to the equation $y=a+bx+c \times \sin (dx+e)$, shown as solid curve, gives an annual growth length of $33.1(^{+3.0}_{-2.5})$ cm/yr ($a=-158$, $b=-0.7265$, $c=8.69$, $d=-0.196$, $e=3.98$, $R^2=0.53$, $n=34$). A complete year's signal could not be measured because the hair length is limited to 20 cm, limiting the precision of the growth rate determination.

Fig. 7. δD values of a modern horse tail hair as a function of distance along hair. The best fit to the equation $y=a+bx+c \times \sin (dx+e)$, shown as a solid curve, gives an annual growth length of 46 cm/yr ($a=-93$, $b=0.27$, $c=7.08$, $d=0.137$, $e=8.73$). There is a slight increase in the δD values of the horse over the course of the year, unrelated to frequency.

between altitude and isotopic composition of meteoric water [24]. On the basis of Fig. 5, it appears that the Incan boy lived at an altitude of ∼1600 m above sea level for the last year prior to his death. The δD values of three principle rivers in the study area have been measured [23]. The δD values of waters of the Mendoza and Tunuyán rivers, both derived from high altitude in the Cordillera de los Andes, are −140 and −127‰, respectively. The δD values from the Pampa de Canota River are −47. It appears, therefore, that the Incan boy received his water principally from the lower elevation sources.

Had he moved to higher altitudes within the last several weeks of his life, the δD values of his latest grown at the base of the scalp hair would have been closer to the −127 to −140‰ measured from the Mendoza and Tunuyán Rivers. Instead, δD values show a smooth sigmoidal pattern along the entire length with no anomalies, suggesting that movement to high altitude occurred very shortly before his death.

4.4.2. Woolly mammoth

Little is known about this hair sample other than it was found at the Yuribei Locality on the Gidan Peninsula, West Siberia. It was donated to Smithsonian Institution by a Russain visitor 20 years ago (R. Purdy, pers. comm., 2002). The δD values along the length of the hair cover a surprisingly restricted range of −157 to −168‰ (Fig. 6), far less than the nearly 60‰ variations seen in the Inca mummy's hair. Modern seasonal variations in δD values of modern Western Siberian precipitation are greater than 50‰ [13]. The hydrogen intake of the mammoth (food+water) should be strongly related to the δD value of local meteoric water. The lack of strong seasonal variation in the mammoth hair δD values can be explained if the individual migrated to
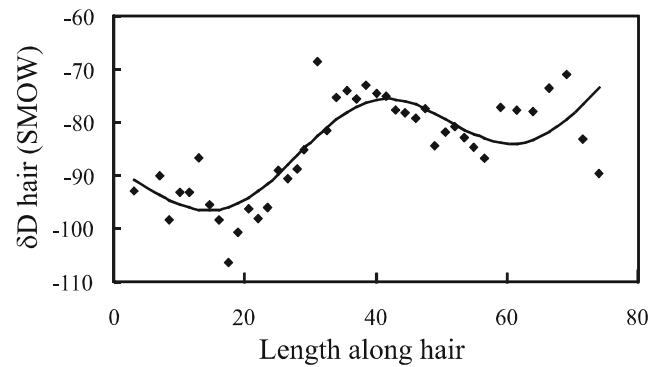
more southern latitudes in winter, thereby avoiding exposure to seasonal isotopic extremes. A regional-climatic 50‰ summer–winter ΔD variation would thus not be fully recorded in an animal that seasonally migrates on the order of 1000 km. Alternatively, the range in δD values of regional meteoric water during the Mammoth's lifetime could have been smaller than today, or the data have undergone diagenesis and reequilibrated to a nearly constant value, although this seems unlikely given the sub 0 °C conditions of preservation.

There is less than a single year recorded in the 20 cm mammoth hair. A best fit of a sinusoidal function $y=a+bx+c \times \sin (dx+e)$, where $y=\delta D$, $x=$distance (Fig. 6), suggests a growth rate of $33.1(^{+3.0}_{-2.5})$ cm/yr, faster than for human hair. For comparison, we measured the hair growth rate for a domestic horse to be 46 cm/yr (Fig. 7). Thus, the growth rate of woolly mammoth hair is intermediate between human head hair and horse tail hair.

5. Conclusions

- Only about one third of the hydrogen in human hair keratin is biochemically and isotopically derived from drinking water, whereas the remainder derives from food hydrogen.
- In most archeological hair, a close link will exist between δD values of hair, food, and local meteoric water, but specimens from environments with isotopically diverse food and/or water sources (e.g., a marine-based dietary component, or in cultures with access to food and water from sharply contrasting altitudes), the δD value of hair may be partially decoupled from local meteoric water influence. In other words, the $\Delta\delta D_{\text{water–hair}}$ is small, so that δD_{hair} values are similar to those of local meteoric water in humans and terrestrial mammals that do not migrate.

- Isotopically exchangeable hydrogen in human facial hair amounts to approximately 10% of total hydrogen in hair. Exchange with atmospheric water vapor or liquid water at room temperature occurs over a period of hours to days. Accurate determination of δD_{hair} must reproducibly equilibrate, and thus isotopically control exchangeable hydrogen.
- Hydrogen isotope ratios of hair offer an opportunity to resolve weekly to daily isotopic signals in mammals directly relating to food and water intake, and thus providing a link to paleoenvironmental and paleodietary conditions.
- Hair tends to be well preserved in many archeological sites and fossil specimens, especially under dry and permafrost conditions, but also in bogs and hypersaline environments. Although stable isotope determination is a destructive analytical technique, modern mass-spectrometer instruments as the one described in this study yield high-precision data while consuming sub-milligram samples.

Acknowledgements

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